

### INTERPRETATION

**Calculation of the Ratio**

- **APP 1-2-9-11**
  
  Calculate the mean (M) of ODs obtained for the APP 1-9-11 positive control (M OD<sub>APP 1-9-11</sub>), the APP 2 positive control (M OD<sub>APP 2</sub>), the negative control and each sample (M OD<sub>S</sub>). Then, calculate the mean of the means of the positive controls (M OD<sub>APP 1-9-11-2</sub>). To obtain ratio, divide each sample’s mean OD by the mean of the means of the positive controls (M OD<sub>APP 1-9-11-2</sub>).

  **Example**
  
  - OD<sub>APP 1-9-11</sub>: 1.250 et 1.200 ; M OD<sub>APP 1-9-11</sub> = (1.250 + 1.200)/2 = 1.225
  - OD<sub>APP 2</sub>: 1.100 et 1.200 ; M OD<sub>APP 2</sub> = (1.100 + 1.200)/2 = 1.150
  - M OD<sub>APP 1-9-11-2</sub> = (1.225 + 1.150)/2 = 1.187
  - OD<sub>S</sub> = 0.800 et 0.850 ; M OD<sub>S</sub> = (0.800 + 0.850)/2 = 0.825
  - Ratio<sub>S</sub> = 0.825/1.187 = 0.695

- **APP 4-5-7**
  
  Proceed as for plates APP 1-2-9-11

- **APP 3-6-8-15**
  
  Calculate the mean (M) of ODs obtained for the APP 3-6-8-15 positive control (M OD<sub>3-6-8-15</sub>), the negative control and each sample (M OD<sub>S</sub>). To obtain ratio, divide each sample’s mean OD by the mean value of the positive control (M OD<sub>APP 3-6-8-15</sub>).

**Validity Criteria**

The following criteria must be met in order to validate the analysis:

- Negative control ratio must be less than 0.15.
- Mean of each of the different positive controls ODs must be greater than 0.70.

**Interpretation:**

<table>
<thead>
<tr>
<th>APP 1-2-9-11</th>
<th>APP 3-6-8-15</th>
<th>APP 4-5-7</th>
</tr>
</thead>
<tbody>
<tr>
<td>negative</td>
<td>ratio &lt; 0.33</td>
<td>ratio &lt; 0.30</td>
</tr>
<tr>
<td>suspect</td>
<td>0.33 ≤ ratio &lt; 0.50</td>
<td>0.30 ≤ ratio &lt; 0.50</td>
</tr>
<tr>
<td>positive</td>
<td>ratio ≥ 0.50</td>
<td>ratio ≥ 0.50</td>
</tr>
</tbody>
</table>

In case of suspect or positive result, it is recommended to test the samples against the relevant serotypes using kits specific for these serotypes (ie. Swinecheck® APP 1-9-11, APP 2, APP 3-6-8, APP 4-7, APP 5).
**MATERIAL**

**Components**
- 12 strips of 8 wells coated with APP 1-2-9-11 antigens
- 12 strips of 8 wells coated with APP 3-6-8-15 antigens
- 12 strips of 8 wells coated with APP 4-5-7 antigens
- Ready-to-use positive controls of APP 1-9-11, APP 2, APP 3-6-8-15, APP 4-7, and APP 5
- Ready-to-use negative control
- Concentrated conjugate
- Concentrated wash solution (10X)*
- Ready-to-use substrate
- Ready-to-use stop solution*

**Quantity**
- 2.5 mL each APP 4-7, and APP 5
- 7.5 mL
- 75 μL
- 4 X 100 mL
- 70 mL
- 70 mL

* Crystals may form when stop solution and concentrated wash solution are kept at 2-7°C. This will not affect the efficiency of the products. However, it is very important that crystals are completely dissolved before using solutions. To dissolve crystals, simply bring the solutions to room temperature and agitate.

**Materials Required but not Provided:**
- Purified water
- Adjustable single- and multi-channel micropipettes
- Single-use micropipette tips
- ELISA microplate washer (optional)
- Test tubes for sample dilution
- ELISA 96-well microplate reader equipped with 405 nm filter
- Containers for preparation of solutions

**PRECAUTIONS**
- For *in vitro* veterinary use only.
- The materials used in this kit must be considered as potentially infectious. Therefore, all waste must be decontaminated before being discarded.
- Do not use the kit after the expiry date indicated on the package.
- Do not mix the reagents from different serial numbers.
- The sensitivity and specificity of this test are guaranteed only if the procedures are strictly observed.
- Do not expose the substrate to either light or oxidizing agent. Always keep the substrate in a plastic container. The substrate and stop solution might cause skin or eye irritation.
- Keep all reagents at 2-7°C and bring to room temperature before use.

**EXECUTION**

**A. Preparation of Wash Solution**
After homogenizing the concentrated wash solution (no evidence of crystals), dilute at 1/10 with purified water (e.g., 100 mL 10X concentrated wash solution in 900 mL purified water for each plate). Once diluted, the solution (1X) is stable for 1 week at 2-7°C.

**B. Sample Preparation**
It is strongly recommended to test the samples and controls as duplicate. Dilute porcine serum samples in 1X wash solution (see section A) at 1/200 (e.g., 4 μL sample in 796 μL 1X wash solution). Make sure you use a new tip for each sample. Also make sure each dilution is properly mixed before being distributed into the wells.

**C. Conjugate Preparation**
Dilute the conjugate with 1X wash solution (see section A) according to the dilution indicated on the Quality Control Certificate. Dilute conjugate a few minutes prior its use and always prepare a fresh solution.

**D. Test Procedures**
Bring all reagents to room temperature and mix well manually before use. For each plate used:
1. Make a schematic representation of the plate and the distribution of controls and samples.
2. Dispense 100 μL of each ready-to-use positive control into two wells:
   - For APP 1-2-9-11 plates, use controls APP 1-9-11 and APP 2
   - For APP 3-6-8-15 plates, use control APP 3-6-8-15
   - For APP 4-5-7 plates, use controls APP 4-7 and APP 5
3. Dispense 100 μL ready-to-use negative control into two wells of each of the plates.
4. Dispense 100 μL diluted samples (see section B) into two wells of each of the plates APP 1-2-9-11, APP 3-6-8-15, APP 4-5-7.
5. Incubate at 23 ± 2°C for 30 minutes.
6. Wash each well 5 times with 300 μL 1X wash solution (see section A). Throw away all liquid contained in the plate after each wash. After the last wash, dry the plate by tapping it on absorbent paper.
7. Dispense 100 μL ready-to-use negative control into two wells of each of the plates.
8. Incubate at 23 ± 2°C for 30 minutes.
10. Dispense 100 μL ready-to-use substrate into each well.
11. Incubate, away from light, at 23 ± 2°C for 20 minutes.
12. Dispense 100 μL ready-to-use stop solution into each well.
13. Measure optical densities (OD) at 405 nm. If the microplate reader is equipped with a reference filter, set it at 490 nm. The reading should be done no later than 15 minutes after the addition of the stop solution.
14. Calculate the results.